Reference: *Biol. Bull.*, **144**: 567–579. (June, 1973)

THE NEUROMUSCULAR BASIS OF COXAL FEEDING AND LOCOMOTORY MOVEMENTS IN *LIMULUS* [1]

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The control of movements by central and reflex integrative mechanisms has been studied in a number of arthropods (for reviews see Kandel and Kupfermann, 1970, and Evoy and Cohen, 1971). Until recently, little work has been done on control of movements in *Limulus*, despite their widespread use in other areas such as visual physiology. *Limulus polyphemus*, the horseshoe crab, is large, hardy, and tolerant of experimental manipulation. It also possesses several stereotyped behavior patterns that are amenable to analysis of their neural control, including rhythmic gill ventilation (Fourtner, Drews, and Pax, 1971; Wyse, 1972) and tail spine rotation (Silvey, 1971; Eagles, 1971, 1973). The patterns considered in this study are the rhythmic coxal movements associated with feeding and locomotion.

Limulus feeds by rhythmic transverse abduction and adduction of the segments of the walking legs. Each coxa, which is elongated dorsoventrally, moves in an arc about a dorsolateral pivot. Gnathobase spines, directed inward, serve to shred particulate food and push it into the mouth, which is in the middle of the legs. Opposite legs move in phase and adjacent legs move out of phase, so that both first and both third legs move inward while both second and both fourth legs move outward, and vice versa. Both these chewing movements and the anatomy of the coxal muscles mediating them have been described (Manton, 1964). The present study shows that for an individual leg, both the movements and the underlying muscle actions are more complex than indicated by Manton, and describes the patterns of motor output controlling different types of rhythmic feeding movements.

Materials and Methods

Specimens of *Limulus polyphemus* (L.) were obtained from the Marine Biological Laboratory, Woods Hole and were maintained in a 150 gal recirculating sea water system. Animals were usually placed ventral side up in air for observation and recording of feeding activity. They were fed small pieces of frozen ocean perch (*ca.* 0.5–1 g, thawed to room temperature) to elicit feeding movements.

Muscle electrical activity was recorded chronically from intact, restrained animals with insulated 40-gauge stainless steel wires from which the insulation had been removed from the terminal 1–2 mm. These electrodes were inserted through fine holes in the cuticle into the underlying muscles and held in place with Eastman 910 adhesive and vinyl adhesive tape. The origins of the tergocoxal muscles (25–29 in Fig. 1) are clearly visible on the dorsal prosoma of most animals, allowing electrode placement at the origins of these muscles. For the four ventral

[1] This work was supported by PHS Grant NS 08869 to G. A. Wyse.

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plastrocoxal muscles, each electrode was inserted through the articular membrane between the legs, into the muscle near its coxal insertion. Indifferent electrodes were placed in the prosoma near its lateral margin. In all cases the electrode positions were confirmed by dissection after the experiment.

To clarify patterns of coxal chewing movements, unrestrained *Limulus* were fed, ventral side up in air, and their activity was photographed with a Bolex Rex 16 mm cine camera at 18–24 frames/sec. Frame-by-frame analysis of individual chewing cycles was performed with a time-motion study projector. Coxal movements were also recorded along with muscle activity, by coupling the gnathobase through a weak spring to a Grass strain gauge.

Results

Chewing patterns

The rhythmic chewing movements of a coxa sometimes consist of simple alternate abduction and adduction about the dorsolateral pleurocoxal pivot, as described by Manton (1964). However, we noted that similar in-and-out coxal movements were used to egest food such as fish that was several days old, a finding which argued for greater complexity of the coxal movements. Further observation showed that in most normal chewing, the coxa did not trace a simple arc about its pivot, but instead took an oval path of: depression-adduction-elevation-abduction (Fig. 1). This coxal movement pattern of *ingestive chewing* then resembled a plot of a hysteresis loop rather than a simple arc. *Egestive chewing* resulted from the reversed sequence of: elevation-adduction-depression-abduction.

This "hysteresis" in the chewing cycle requires movement of the pleurocoxal pivot. The coxa articulates with a Y-shaped pleurite, which in turn is set in pliable pleural cuticle. The pleurite serves as a rather firm pivot against anterior-posterior movement, but is relatively free to move dorsolaterally. Such movements of the pleurocoxal pivot are observed during both ingestive and egestive chewing, but the inaccessibility of the pivot and movements of distal leg segments make them difficult to quantify.

Anatomy of coxal muscles

The coxal muscles have previously been described (Lankester, Benham and Beck, 1885; Manton, 1964). Although their actual arrangement is more complicated than previously indicated, only a brief outline of the anatomy will be given here. Each of the first four walking legs has nine coxal muscles (the fifth leg is only tonically active during chewing, and was not examined). The third walking legs were selected for detailed study, but there is little difference in muscle anatomy among the first four legs. Five of the nine coxal muscles originate dorsally on the carapace (tergocoxals), and four (plastrocoxals) originate on the endosternite, a central cartilage-like mass derived from fused muscle tendons (*en* in Fig. 1.). The position of the endosternite can be considered fixed over the course of several coxal chewing cycles; since adjacent legs move out of phase with each other, any movement of the endosternite cannot contribute to a coxal movement cycle.

The muscles are numbered according to Lankester *et al.* (1885). In their description, serially homologous tergocoxal muscles bear the same number, but

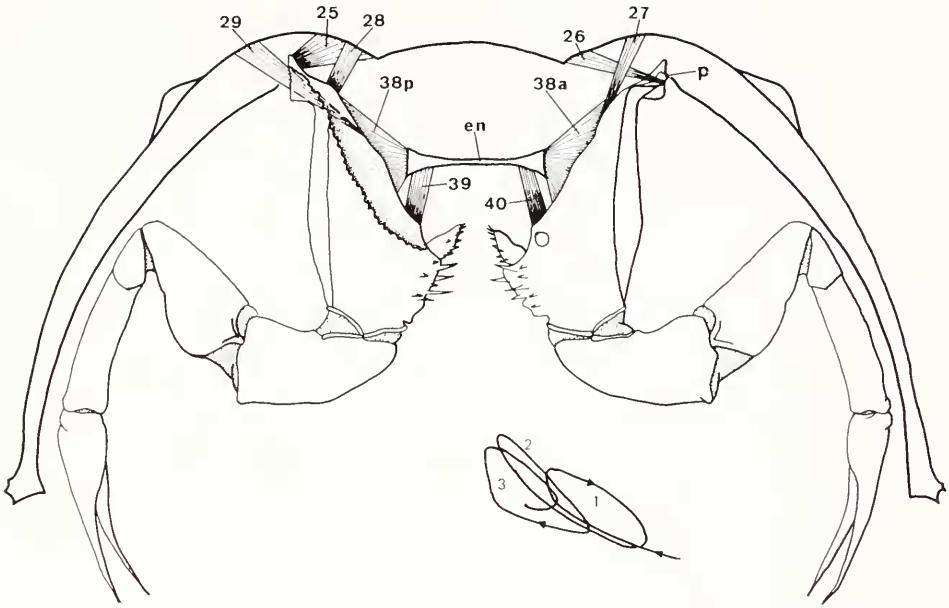

FIGURE 1. Anterior view of a *Limulus* at the level of the third walking legs. On the right are shown the muscles inserting on the anterior face of the coxa of the third leg. On the left, the anterior face and muscles have been cut away, exposing the muscles inserting on the posterior face. Muscles are numbered according to Lankester, Benham, and Beck (1885). Abbreviations are: en = endosternite, p = pleurocoxal pivot. The diagram under the legs shows three consecutive cycles of chewing (labeled 1–3) of a leg. The path of a coxal gnathobase spine was traced from frames of a cine film. Note that the paths are oval rather than simple arcs. The net inward movement of the coxa over several cycles resulted in part from decreased resistance to adduction as the food, a piece of fish, was swallowed. The scale of the path is larger than that of the diagram of the animal above.

serially homologous plastrocoxals bear different numbers for each pair of legs. The numbers used here are for the third legs. The tergocoxals 25, 28, and 29 and the plastrocoxals 38p and 39 insert on the posterior side of the dorsomedial coxal margin (Fig. 1). The tergocoxals 26 and 27 and the plastrocoxals 38a and 40 insert on the anterior side of the margin. Several of the muscles have an anterior or posterior component to their action, not visible in the transverse view of Figure 1. As indicated in Figure 2, 25, 38p and 38a originate anterior to the leg on which they insert, while 26, 39, and 40 originate posterior to their insertions.

Muscle activity during normal ingestive chewing

Activity was recorded from 4–6 muscles at a time, usually from third walking legs, in over twenty intact specimens of *Limulus*. The degree of "hysteresis" in normal chewing was not quantified in recording, but was frequently noted to be present in slight or moderate degree. Representative records of muscle activity during normal chewing are shown in Figure 3. Most of the muscles were active during instroke (adduction, represented in all records by upward deflection of the monitor).

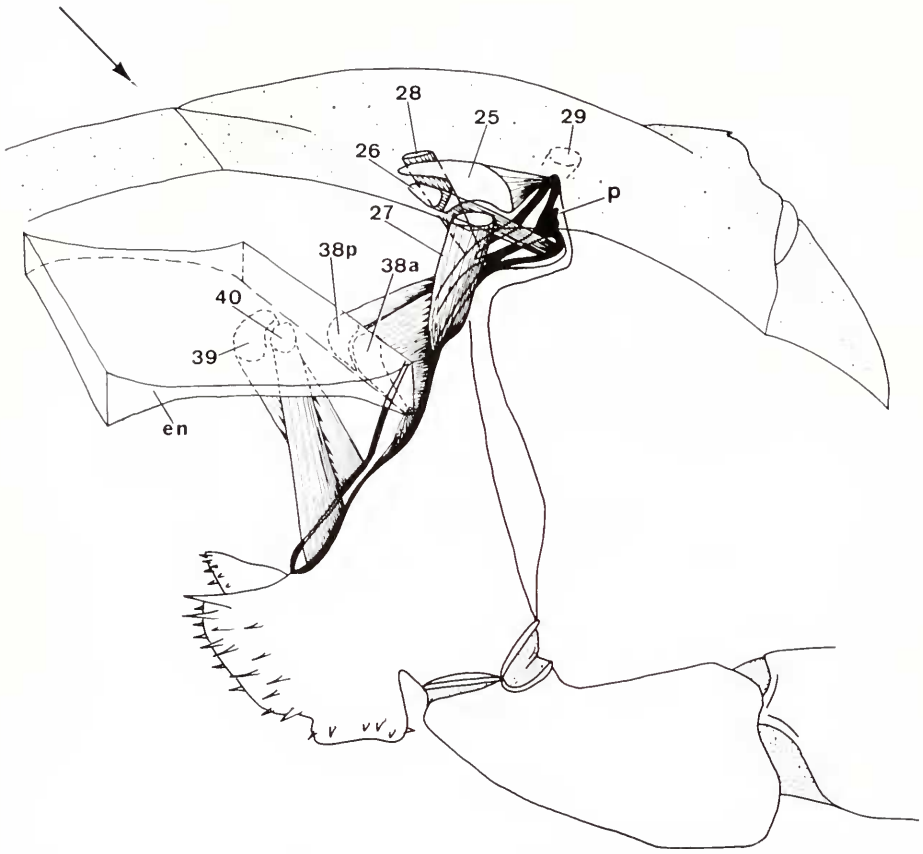

FIGURE 2. Perspective view of the third left coxa, showing muscle attachments. The arrow indicates anterior direction. Note that the directions of muscle action are oblique rather than simply transverse. Abbreviations are: en = endosternite, p = pleurocoxal pivot.

Muscles 39, 40, 38p and 38a (the four ventral plastrocoxal muscles) were always active during instroke (Fig. 3A), although the amplitude in 38p varied. Muscle 40 frequently terminated activity before the end of instroke. Activity of all four usually started 0.1–0.2 sec before the onset of observable inward movement. In some preparations a second period of synchronous activity was present in all four plastrocoxal muscles during outstroke (Figs. 3A, 4). Figure 4 shows the two phases of plastrocoxal activity at a faster time base. Activity during instroke was biphasic negative-then-positive, and was not synchronous between the four muscles. In contrast, the activity during outstroke was highly synchronous for all four muscles, and was biphasic positive-then-negative. Since the adjacent legs move out of phase with the recorded leg, this activity might have resulted from electrode pickup from their adjacent muscles. However, 38a would pick up from the second leg and 38p from the fourth leg, so that synchronous activity would be highly unlikely. The synchronous activity during outstroke did not correlate with

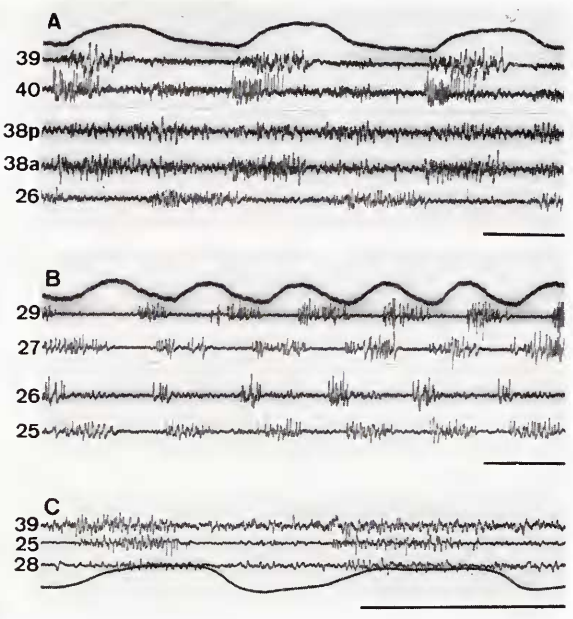

FIGURE 3. Electromyograms of coxal muscles during normal chewing. Upward displacement of the monitor indicates inward coxal movement (top trace in A and B; bottom trace in C); further explanation in the text. All time marks 2 sec.

tension development in the muscles, an observation confirmed by recording tension development in individual muscles, the insertion of which had been dissociated from the coxa. Such muscles developed tension during instroke only and relaxed during outstroke-phase synchronous activity. It seems most likely that this synchronous activity represents the action of a peripheral inhibitor axon common to all four plastocoxal muscles. Although similar evidence for peripheral inhibition has been reported in lobster swimmeret muscles (Davis, 1969), intracellular recording from the plastrocoxal muscles will be required to test the hypothesis of a common inhibitor.

The activity of the five tergocoxal muscles is more diverse (Fig. 3A-C). Muscles 25 and 28 were only active during instroke, although 25 could tail over into the beginning of outstroke. The only muscle with activity consistently during outstroke was 26. Its activity was usually confined to the latter part of outstroke; the record in Figure 3B is more typical than that in 3A in this respect. During weak chewing, there was little or no activity in 25 and 26; they were recruited during stronger cycles.

The two muscles directed nearly radially to the axis of coxal movement had the most variable activity. Muscle 27 was active during instroke, sometimes continuing through early outstroke. Muscle 29 was extremely variable and could act during instroke, outstroke, or both (Figs. 3B, 5). Three patterns of activity were observed most frequently: (1) during instroke, (2) during both late outstroke and late instroke (in two discrete phases), and (3) through late instroke and early

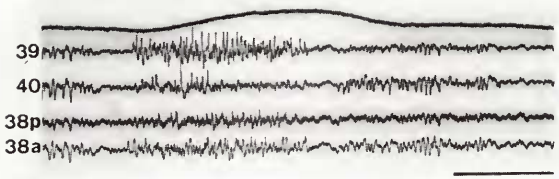

FIGURE 4. Electromyograms of plastrocoxal muscles during normal chewing, showing asynchronous activity during instroke (up) and synchronous activity during outstroke (down); further explanation in the text. Negative is up for all electrodes; time mark 1 sec.

outstroke, lagging 90° or more in phase behind the instroke. During normal chewing, 29 never appears active during the beginning of instroke. Figure 6 summarizes the characteristic patterns of activity of coxal muscles during ingestive chewing.

Muscle activity during egestive chewing

When experimental animals were fed fish that was several days old, the pattern of egestive chewing often resulted, in which the coxal gnathobases moved in the sequence medial-ventral-lateral-dorsal. Representative records of coxal muscle activity recorded during such a pattern are shown in Figure 7, and a summary of the activity patterns in all such cases is shown in Figure 8. Most of the muscles had patterns of activity nearly indistinguishable from those during normal chewing. The only muscles whose pattern changed markedly were the radially directed tergocoxals, 27 and 29. Muscle 29 was active during late outstroke, often with a secondary burst early in instroke. Activity in 27 started in mid-outstroke and continued until late instroke. Taken together, activity in the radially directed muscles 29 and 27 phase-led instroke during egestive chewing (Fig. 8). In contrast, activity in these two muscles tended to phase-lag behind instroke during normal ingestive chewing (Fig. 6). The significance of these changes in phase is discussed below.

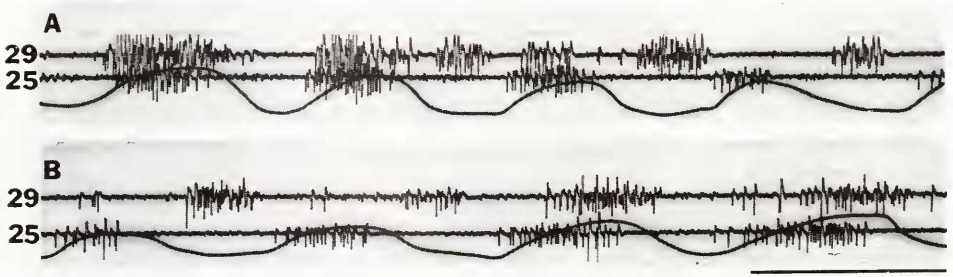

FIGURE 5. Electromyograms of tergocoxal muscles 29 and 25, showing extreme variation in the activity of 29. The two records are in sequence, with two cycles omitted between them. Muscle 29 acted during instroke at the beginning and end of the records, but acted during both phases or during outstroke in intervening cycles; time mark 2 sec.

Coxal promotor and remotor movements

The activity of muscles during the coxal promotor-remotor swing (Manton, 1964) was examined for comparison to activity during chewing. The animals were allowed to walk over a glass plate in air. In contrast to transverse rotation about the dorsolateral pivot in chewing, the anterior-posterior coxal swing must involve two functional pivots. In addition to the fixed dorsolateral pivot, a second indistinct point near the gnathobase at the ventromedial coxal margin is also

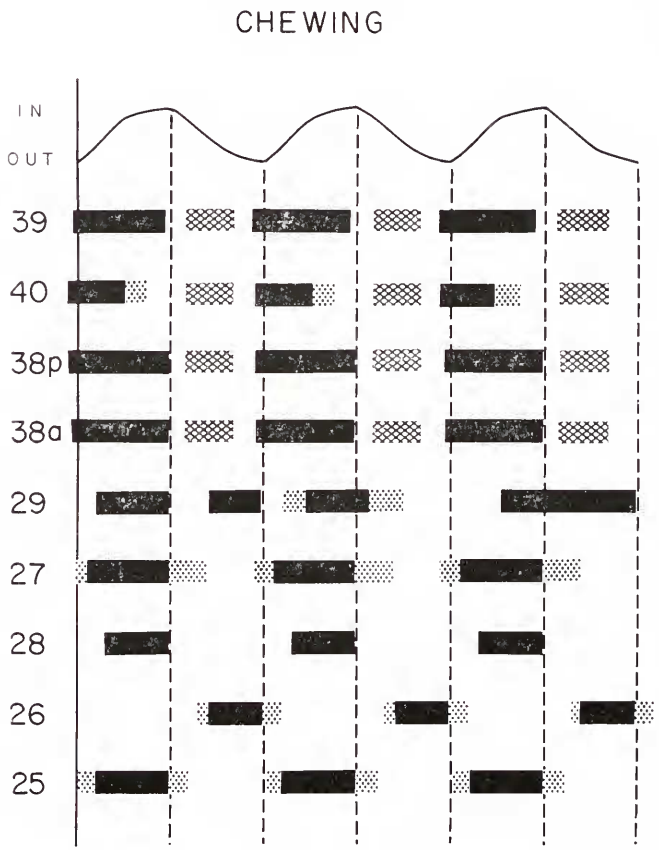

FIGURE 6. Summary of muscle activities of all preparations during normal ingestive chewing. Black bars indicate the period during which a muscle was usually or always active. Dotted areas indicate periods when muscle was sometimes active. Crosshatched areas indicate periods of synchronous, positive-going activity (inhibition?) in 38a, 38p, 39, and 40. For 29, the three common activity patterns are shown.

stationary. The coxa then swings like a door around two hinges. The general pattern of muscle activity during this swing was as follows (see Figs. 9 and 10): muscles 26, 27, and 38a, which attach to the anterior face of the coxa, were active during forward movement (promotion). Muscles 25, 38p, and 39, which attach

to the posterior coxal face, were active during backward movement (remotion). Muscle 29 (recorded once) was inactive or weakly active during remotion; activity in 28 during coxal swing was not recorded. Muscle 40, although inserted on the anterior coxal face, was active during remotion. Secondary activity was sometimes seen in 38a, 26, and 27 during remotion. The secondary activity in 26 and 27 sometimes appeared synchronous and could represent either peripheral inhibition or spread of activity from the large adjacent origin of muscle 25. It seems clear, however, that promotion results from inward pull on the anterior coxal margin and remotion results from pulling in on the posterior margin.

Discussion

In the muscular generation of normal coxal chewing movements, the ventral plastrocoxal muscles 38a, 38p, 39, and 40 must serve as the main adductors. They

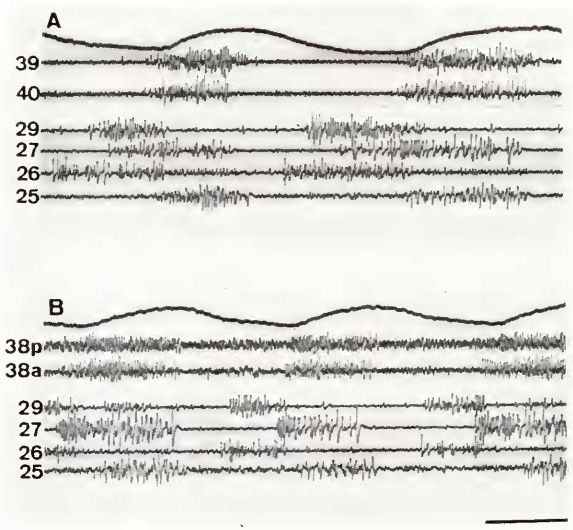

Figure 7. Electromyograms of coxal muscles during egestive chewing; upward deflection of monitor = inward movement; time mark 2 sec.

are the muscles with the greatest leverage around the pleurocoxal pivot (Fig. 1), and their combined activity appears to be an invariant correlate of adduction.

Manton (1964) in her anatomical study of coxal muscles, concluded that the tergocoxal muscle 25 actively abducted the coxa. She felt that 25 inserted dorsal as well as posterior to the pleurocoxal pivot, and hence could abduct. She rejected the possibility of abduction by passive elasticity, feeling that the arthroidial cuticle was not sufficiently elastic. Our results clearly show that 25 is active during adduction only, never during abduction. The only muscle consistently active during abduction was 26. It is anatomically unlikely that 26 could abduct. It is the main promotor during coxal swinging (see below) and is probably of only secondary importance in chewing, being recruited only with stronger cycles. Direct stimula-

tion of muscle 26 through the recording electrode always produced simple promotion of the coxa, with no abduction. Conjoint stimulation of 26 and 29 (since 29 is sometimes also active during abduction) likewise produced simple promotion. It seems clear that none of the coxal muscles are abductors. Abduction must then result from passive forces, perhaps by cuticular elasticity aided by active inhibition of adductors. Lateral displacement of the coxa by adduction of adjacent coxae in the restricted space around the mouth could also aid in passive abduction.

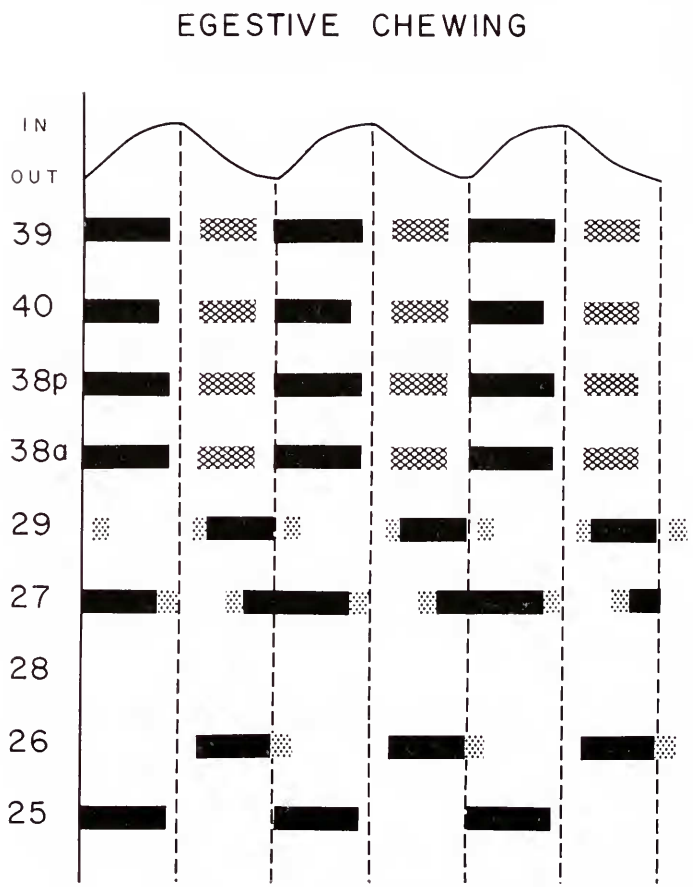

FIGURE 8. Summary of muscle activities during egestive chewing. Symbols are the same as in Figure 6. Activity patterns are similar to those in normal chewing (Fig. 6), except that 29 and 27 tend to phase-lead instroke.

Snodgrass (1950) hypothesized that arthropod jaws evolved from leg coxae, with a primitive condition of direct transverse jaw movements worked by adductor muscles. Abductor muscles were considered to be absent, with passive abduction resulting from cuticular elasticity. Manton (1964) opposed this view, arguing that Snodgrass' hypothetical ancestor did not resemble any living arthropod, and that passive abduction was undemonstrated and unlikely. Manton (1964) concluded

that various arthropod jaw mechanisms had evolved independently, and considered her study to support a hypothesis of polyphyletic origin of arthropods (see Tiegs and Manton, 1958). We find no evidence for a functional abductor muscle in the transverse chewing movements of *Limulus*. Therefore one of Manton's objections to Snodgrass' hypothesis is not verified, but implications concerning the evolution of arthropod groups remain unclear.

In the muscular generation of the promotor-remotor coxal swing of walking, the dorsal tergocoxal muscles must predominate. In agreement with Manton (1964) we conclude that muscle 26 is probably the main promotor, while muscle 25 is probably the main remotor. This conclusion is based in part on anatomical arrangement of the muscles, which allows maximum leverage anterior and posterior to the pleurocoxal pivot. Furthermore, on direct electrical stimulation, 25 always produced simple coxal remotion, while 26 always produced simple promotion. Stimulation of 27 and 28 produced variable mixtures of weak promotion or remotion

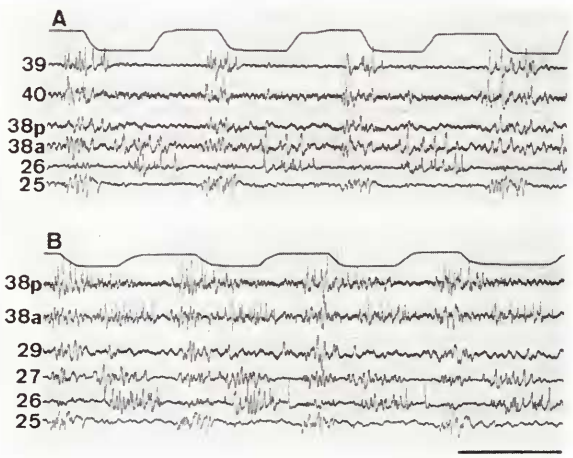

FIGURE 9. Electromyograms of coxal muscles during the coxal swing of promotion and remotion. The movement monitor (top traces, forward promotion is up) was hand-controlled, and gives only an approximation of the time of coxal movement; time mark 1 sec.

and adduction. The plastrocoxal muscles may contribute to promotion and remotion, but their major role is probably stabilization of the ventromedial coxal margin. During walking in aquaria the gnathobases are held rather laterally. Thus the activity of the plastrocoxal adductors must not generate a great degree of shortening of the muscles.

The larger remotor muscle 25 pulls the posterior dorsolateral corner of the coxa both dorsally and medially (see Figs. 1 and 2). The medial component of this force would displace the whole ventral portion of the coxa forward as well as remoting. The combined action of muscles 39 and 40, both of which pull the ventral part of the coxa back as well as in, and both of which act during remotion, would tend to oppose the forward displacement of the coxa and stabilize remotion.

The circularity or "hysteresis" of coxal movements during ingestive and egestive chewing is considered to result from action of muscles 29 and 27. The presence

of any circularity in the arc of transverse movement about the dorsolateral pivot requires that the pivot move on an axis radial to the arc. Muscles 27 and especially 29 are radically directed (Fig. 1) and can act as *pivot shifters*. Of the three relatively stable patterns of action of muscle 29, the one likely to produce the greatest degree of "hysteresis" is with 29 activity phase-lagging 90° or more behind adduction. The coxa would be adducted inward, and then pulled dorsolaterally during late

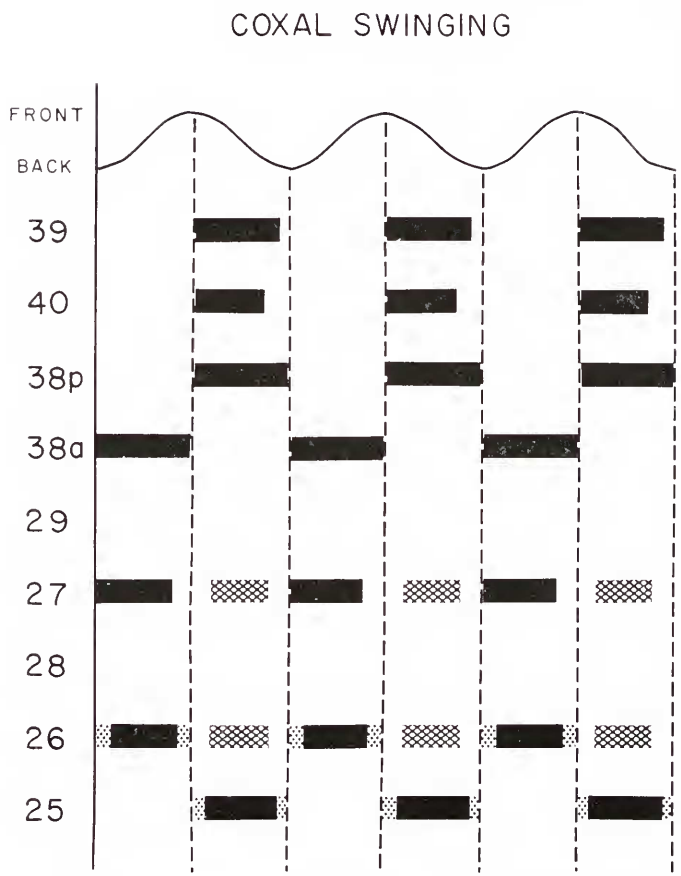

FIGURE 10. Summary of muscle activities during promotor-remotor coxal swinging. Symbols are the same as in Figure 6. Activity of 28 was not recorded during swinging; activity in 29 was recorded in only one preparation and thus could not be characterized reliably.

instroke and much of outstroke. Therefore, the outstroke path would be dorsolateral to the instroke path, producing the sequence medial-dorsal-lateral-ventral.

By the same argument, the pattern of activity of muscles 29 and 27 during egestive chewing is sufficient to produce circularity in the opposite direction. Taken together, the radial muscles phase-lead instroke by about 90°, producing a dorsolateral force vector during late outstroke and early instroke. The resulting movement sequence is the medial-ventral-lateral-dorsal pattern of egestion.

The difference between chewing resulting in ingestion and chewing resulting in egestion is thus a result of a phase shift in the action of two muscles within an otherwise rather stable pattern of muscle activities. The sensory information underlying this phase shift presumably comes from the same gnathobase chemoreceptors (Barber, 1956; Barber and Hayes, 1963) that trigger all chewing sequences. The central integrative mechanisms underlying the chemosensory control of this phase shift are interesting subjects for further research.

Summary

1. Transverse feeding movements of a *Limulus* leg coxa can trace a simple repeating arc of abduction and adduction around a dorsolateral pivot, or may take an oval path of depression, adduction, elevation, and abduction. This ingestive chewing path and the reverse sequence mediating egestion both require movement of the pivot.

2. The actions of the nine coxal muscles were determined by chronic electromyogram and movement recordings in intact animals. In all feeding patterns most muscles act during adduction, the four ventral plastrocoxals being the main adductors. None of the muscles actively abduct the coxa.

3. In the promotor-remotor coxal swing of locomotion the dorsal tergocoxal muscles predominate, although the plastrocoxal muscles are also active. Muscles attached to the posterior margin of the coxa are active during remotion, and those on the anterior margin are active during promotion (except muscle 40, which is active during remotion).

4. The oval paths characteristic of ingestive and egestive chewing result from action of muscles 29 and 27. These muscles act radially to the arc of adduction-abduction and displace the pivot of that arc dorsolaterally. When activity in these muscles phase-lags adduction, the path of ingestive chewing results. When their activity phase-leads adduction, the reverse sequence of egestive chewing is produced. Thus a major behavioral alteration results from phase-shifting the action of two muscles in an otherwise stable pattern of motor output.

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